STARVATION AND THE RESISTANCE OF FISHES TO LACK OF OXYGEN AND TO KCN.

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I. Introduction.

The following paper is a report on experiments that were carried on at the University of Chicago during the fall and winter of the years 1913 and 1914. The object was to determine the effect of starvation upon the rate of metabolism in fresh-water fishes. The work is still in progress, but it has seemed best to record briefly at this time some of the results thus far obtained.

II. Material and General Methods.

Practically all of the experiments reported here were performed with the rock bass (*Ambloplites rupestris* Raf.). To confirm the apparent similarity of the effects of the low oxygen and the KCN treatments, experiments with tadpoles and three or four other species of fishes were performed.

All of the animals used were collected in the streams and ponds in the vicinity of Chicago. The collections were made during the months of October, November and December. The animals were brought into the laboratory at once and with a minimum amount of handling. They were weighed immediately and those that were to be starved were placed in compartments in aquaria through which tap water coming from Lake Michigan was flowing. No attempt was made to remove the plankton from this water and if the starving animals secured food from it the amount was far from sufficient to meet their normal needs, for the loss of weight due to starvation proceeded uniformly with the exception of two weighings (see Table I.) up to the death point. The aquaria were kept free of plant growth and no food of any kind was given the animals.

After the initial weighing the starving fishes were reweighed at gradually increasing intervals. Thus at first they were weighed every other day while later a week or ten days was allowed to elapse between weighings.

It was noted that the weight of the fishes varied slightly with the temperature of the water in which they were confined just previous to being weighed. A fish which weighed 32 grams at 5° C. weighed 32.1 grams after being placed in 12° water for 15 min. Another fish weighed 71.8 grams at 5° and 72.1 grams at the end of 15 min. in 12° water. This temperature factor was eliminated by weighing the fishes rapidly when they were taken from the aquaria for the temperature of the aquarium water changed but slightly after December 1 (varied between 4° and 8° C.).

The rock bass was selected for the experiments herein recorded because this species at the time, was easily caught by seining, in the small streams in the Chicago region; and it had been noted during several years of collecting that the individual fishes seemed to fall into natural size groups which were apparently correlated with age. Five of these groups are readily distinguishable and a sixth is sometimes taken.

The smallest fishes collected weighed from 1–1.5 grams and were evidently the fry of the previous spring. The next larger group averaged from 10–15 grams and included fishes that were probably a little over a year old. The third group weighed from 25–40 grams, the fourth from 80–100 grams and the fifth from 100–125. It is at least possible that these latter groups are made up of fishes that are in their third, fourth and fifth years respectively. Occasionally still larger individuals weighing over 130 grams were taken. Not enough of this group was taken to include it in every experiment and it is probable that fishes of more than one year's growth are included in it. The largest specimen taken weighed 424 grams.

It should be pointed out that not all the fishes collected were easily classifiable into one or another of the above groups for some were taken whose weights placed them on the border line between two groups. This was especially true in the case of groups two and three. However most of the fishes fell readily into one of the five groups and only such fishes were used in the experiments.

III. Experimental Methods.

The resistance experiments were conducted as follows. A starved fish from the experimental aquaria was placed in a large (5-liter) wide-mouth bottle (low oxygen expt.) or in a battery jar (KCN expt.) along with a control fish of the same group. The control fish was selected so that its weight was very near the original weight of the experimental fish. In most of the low oxygen experiments a continuous stream of water flowed through the bottle. This water came from an apparatus that removed all but a trace of the oxygen.[1] The water flowed through the bottle at the rate of 300 c.c. per min.[2] The solutions of KCN were made up by diluting a standard $N/100$ stock solution. The battery jars were covered with glass plates during the experiments.

In all the experiments the control and the experimental fishes were placed in the same bottle or jar. The caudal fin of the control fish was clipped at the top and that of the experimental fish at the bottom. The two were thus easily identifiable. There was no evidence that the clipping of the fins had any effect whatsoever upon the resistance of the fishes. All the control fishes were collected just previous to the performing of the experiments.

IV. Presentation of Data.

1. *Seasonal Resistance of the Fishes.*

During several years' collecting it had been noted that in nature the resistance of fishes to detrimental factors in general,

[1] For description of apparatus see Shelford and Allee, 1913, p. 214.

[2] For complete description of methods of experimentation and recording, see Wells, '13, pp. 325–29.

is lowest in the late summer and highest in the spring. To test these observations experiments with various species of fishes were run in low oxygen water. During the winter when the experiments with starvation were being carried on the seasonal resistance curve of the rock bass was worked out rather fully, for the fall and winter months. This curve is shown in Fig. 1. The solid line represents actual experimental data and the dotted portion, conclusions drawn from field observation and some few resistance experiments performed during the time represented.

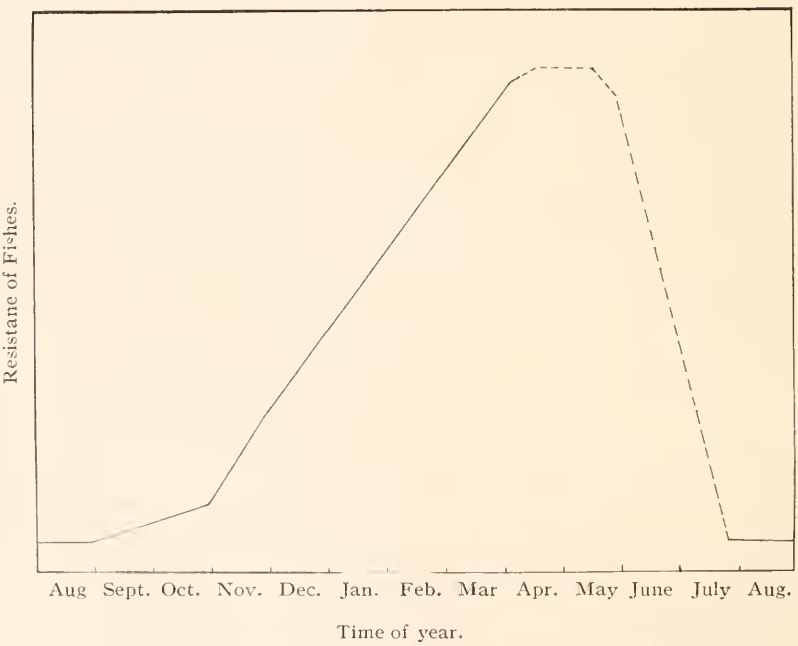

FIG. 1. Curve showing the seasonal resistance of rock bass (*Ambloplites rupestris* Raf.) to lack of oxygen. The curve is based on data secured by testing fishes belonging to group 3 (*i. e.*, fishes weighing from 25–40 grams). However the relative resistance of the size groups remains practically constant throughout the seasons and the curve may be taken as representing the seasonal changes in resistance to low oxygen, of the other groups as well. That part of the curve represented by a solid line is based upon experiments performed during the months indicated. The dotted portion is based upon a knowledge of the August and March resistance, upon field observations, and upon a number of field experiments.

2. *The Process of Starvation.*

One set of six fishes was kept without food until death from starvation resulted. The following table (I.) gives the consecutive weights of the fishes during this time.

Table I.

Showing the regular decrease in weight of starving rock bass (*Ambloplites rupestris* Raf.). The fishes were collected on December 6 and weighed as soon as gotten to the laboratory which was two hours after capture. They were kept without food during the entire period. Numbers at head of column indicate size group to which the individual fishes belonged. Temp. 4°–8° C.

Date of Weighing.	Consecutive Weights of the Fishes in Grams.					
	1.	2.	3.	4.	5.	6.
1913						
Dec. 6	1.85	9.6	38.9	83.5	97.7	133.2
" 8	1.70	8.9	35.7	79.8	89.5	122.1
" 10	1.63	8.8	35.5	76.5	87.0	118.5
" 12	1.58	8.6	35.12	76.0	86.3	117.5
" 17	1.53	8.5	34.75	76.9[1]	85.4	117.3
" 24	1.52	8.3	34.6	75.3	84.7	117.2
" 31	Dead	8.25	34.30	74.5	83.5	116.35
1914						
Jan. 3		8.1	33.9	74.15	82.1	115.9
" 10		7.8	33.5	73.3	79.8	115.66
" 23		7.6	32.7	72.3	77.95	114.43
Feb. 1		Dead	32.45	71.9	77.6	114.1
" 5			32.2	71.7	76.6	113.75
" 8			32.0	71.0	76.3	112.7
" 15			31.6	70.85	76.7[1]	111.3
" 24			31.3	70.6	75.1	111.3
Mar. 8			30.5	69.1	72.5	110.0
" 22			29.8	66.9	69.6	102.6
Apr. 1			Dead			
" 9				64.2	67.2	100.4
" 15				61.7	64.2	96.7
" 17				Dead	62.5	Dead
May 5					Used in Expt.	
Per cent. of weight lost	19	20	23	26	36	27

[1] Increase instead of decrease.

It will be noted from Table I. that the most rapid loss of weight comes within the first day or two when the intestine is cleared of its contents. After this the decrease is gradual, up to the death point.

The table shows two exceptions to the steady falling off in weight. Fish no. 4 lost weight steadily up to December 12, when it weighed 76 grams. On December 17 its weight had increased to 76.9 grams. No way to account for this increase is clear. Some food substance may have gotten into the aquarium and this seems to be the most likely explanation, for one week later the weight had decreased to 75.3 grams. The fish was not dissected and the presence or absence of food ascertained as it was thought best that the series be kept unbroken. Fish no. 5 shows a similar increase in weight on February 8; again in a week the weight fell to a figure below that just previous to the increase and no other rise occurred.

3. *Resistance to Lack of Oxygen.*

Table II. is a summary of experiments performed to determine the resistance of starved fishes to lack of oxygen.

Note that Table II. shows a rapid initial increase in the resistance of the starved fishes to lack of oxygen and that this increased resistance gradually diminishes till after 53 days without food the starving fishes are considerably less resistant than the control fishes. It is also interesting to note that the decrease in resistance following the initial increase proceeds slowly for the first 39 days and then rapidly, till on the fifty-third day the starving fishes show a resistance that is not only lower than that of the control but is also lower than that of fishes of the same size but which had not gone without food for so long a time. The increase in resistance upon the part of the starving fishes is emphasized by the fact that the control fishes show a markedly increased resistance with the progress of the season as shown by the curve (Fig. 1) and the control readings in Table II.

4. *Resistance to KCN.*

When it was found that starvation results in an increase in the resistance of the starving fishes to lack of oxygen, and that this increase is followed later by a rapid and marked decrease in resistance, it was decided to test the susceptibility of fishes of the same species and in similar stages of starvation, to solutions of KCN. In this way the "susceptibility to cyanide" method that has been used so successfully with planaria by Child, was applied to fishes. A comparison of the results obtained by the two methods is interesting in that they show in general the same relationships. Table III. is a summary of experiments performed with starving fishes in *N*/25,000 solutions of KCN.

Table II.

Showing the effect of starvation upon the resistance of rock bass (*Ambloplites rupestris* Raf.) to lack of oxygen. The control fishes were in every case collected just previous to the conducting of the experiment. Control and experimental fishes were placed in the same container. Water containing about .1 c.c. oxygen per liter flowed through the experimental bottle at the rate of 300 c.c. per min. Dying time is indicated in minutes. C = Control; E = Experiment.

No. Days Starved and Date of Experiment.	Serial Number and Weight of Fishes.											
	No. 1. 1–1.5 Grms.		No. 2. 10–15 Grms.		No. 3. 25–40 Grms.		No. 4. 80–95 Grms.		No. 5. 100–125 Grms.		No. 6. 130–200 Grms.	
	C.	E.	C.	E.	C.	E.	C.	E.	C.	E.	C.	E.
Dec. 8.—5 days....	78	75	196	265	324	465	380	732	611	765	564	350
Nov. 25.—39 days..	80	95	180	201	300	410	327	690	540	555	555	690
Dec. 12.—53 days..	75	25	265	160	475	265	865	355	805	315	385	835

Because of the fact that a *N*/25,000 KCN solution is relatively more fatal than water containing practically no oxygen, the figures in Table III. are smaller than those in Table II. and the differences in the resistance of the experimental and the control fishes of correspondingly less magnitude. However Table III. shows the same initial increase in resistance (decrease in suscepti-

Table III.

Showing the effect of starvation upon the resistance of rock bass (*Ambloplites rupestris* Raf.) to *N*/25,000 solution of KCN. Control collected just previous to experiment. Control and experimental fishes in same container. Dying time indicated in minutes. C = Control; E = Experiment.

Date of Experiment and No. Days Starved.	Serial Number and Weight of Fishes.											
	No. 1. 1–1.5 Grms.		No. 2. 10–15 Grms.		No. 3. 25–40 Grms.		No. 4. 80–95 Grms.		No. 5. 100–125 Grms.		No. 6. 130–200 Grms.	
	C.	E.	C.	E.	C.	E.	C.	E.	C.	E.	C.	E.
Dec. 2.—12 days...	65	85	98	95	133	142	162	145	144	152	145	145
Dec. 2.—47 days...	84	97	92	97	128	157	145	164	148	80	140	205
Dec. 7.—52 days...	65	78	128	128	163	128	203	218	163	188	240	233

bility) upon the part of the starved fishes; also as in the low oxygen experiments, after 52 days' starvation, we note that the starved animals are beginning to show a greater susceptibility to the detrimental condition, than is shown by the control. A comparison of the control and experimental animals at this time shows that the experimental fishes in groups 3, 5 and 6 are more susceptible, in groups 1 and 4 they are still less susceptible while control and experiment in group 2 show the same susceptibility. In experiments performed on the sixtieth day of starvation groups 1, 2 and 4 were found to be much more susceptible than the controls.

5. *Comparison of Species.*

Further evidence pointing toward a similarity in the physiological action of lack of oxygen and KCN is suggested by the results of a series of experiments that were conducted with the idea of comparing the effects of the two treatments upon other species of fishes and upon frog tadpoles. Table IV. shows the results of an experiment with KCN in *N*/25,000 concentration and a series of tadpoles and fishes whose relative resistance to low oxygen had been previously determined (Wells, '13). The relative resistance or susceptibility of the species in KCN solution is the same as had already been found for these species in low oxygen water. The tadpoles are markedly most resistant, the catfish is next, the rock bass third and the darter least. In experiments of this kind size differences were eliminated by selecting individuals of a given weight. Thus the weights varied only between 1.5 and 3 grams.

Table IV.

Showing the comparative resistance of different species to *N*/25,000 KCN solution The species are arranged in the order of their increasing resistance. This is the same order that they show in low oxygen water. All the individuals used weighed between 1.5 and 3 grams.

Species.	Dying Time in Hrs. and Min.
Darter (*Etheostoma cœruleum*)	35 min.
Rock bass (*Ambloplites rupestris*)	1 hr. 5 min.
Catfish (*Ameiurus melas*)	27 hrs. 50 min.
Tadpole (*Rana catesbiana*)	208 hrs. 50 min.

V. Discussion.

From the data here presented it is evident that the relative deleterious effects of lack of oxygen and a *N*/25,000 solution of KCN are much the same for the animals in question. This suggests a fundamental similarity in the manner in which these two toxic conditions interfere with the metabolism of organisms. The actual meaning of the similarity is still to be discovered.

The preceding results are of especial interest in their connection with previous work on the metabolism of the lower and the higher animals. Child ('16) has shown that flat worms (*Planaria*) that are morphologically old, if starved, can be made to retrace the metabolic steps taken toward old age and to again attain the morphological appearance and high rate of metabolism that are concomitant in nature with young worms; furthermore these worms are not apparently but *really* young and cannot be distinguished by appearance, physiological activity, or behavior from "naturally" young worms.[1] In this regressive process the planarian becomes smaller, the renewed youth being a result of the tearing down and throwing off of those morphological and physiological structures that slow up cell activity. Rejuvenation is then possible, in the planarian, because of the absence of stable structures such as are present in the higher forms.

Animals with a fixed supporting tissue may perhaps become somewhat rejuvenated by clearing the body cells of obstructions to metabolism but they cannot appreciably diminish the bulk of supporting tissue which they possess. In the mammals, starvation results in emaciation and there is no extensive reorganization such as is found in the flat worms. When a mammal is starved we get a decrease instead of an increase in the rate of metabolism, if we measure this rate by the carbon dioxide output. This depression in the rate of the oxidative processes persists throughout the entire starvation period, there being little evidence at the present time that the metabolism of a starving mammal shows any tendency to increase above the normal rate, even though the starvation period be continued till death results.

In mammals and flat worms then, we have represented, the two extremes of starvation effects so far as rate of oxidations is concerned. In man, starvation effects a depression in rate

[1] It is necessary before a starved planarian will show the same capacity for acclimation to low concentrations of killing agents such as KCN, that it be fed at least once (Child, '16, p. 164).

of metabolism which depression persists from the beginning to the end of the starvation period (Hammarsten, p. 836). In *Planaria*, starvation causes an increase in rate and this increase continues till death occurs. The effect of starvation upon the rate of metabolism in fishes is then of considerable interest for in these forms we have a group that is *structurally* midway between the flat worms and the mammals. It is not surprising therefore that fishes should possess a *physiological organization* that is apparently midway between that of the flat worms and the mammals also. In Table II., p. 447, we saw that the metabolism of starved fishes first shows a depression as in a starving mammal but that it is later accelerated as in starving planarians. The real meaning of this relation is undetermined but it is evident that the fishes resemble the higher forms in the possession of a mechanism which tends to prolong life by decreasing the rate at which the reserve tissues are used up. This is the only method possessed by mammals for withstanding starvation but fishes are also apparently capable of a certain degree of reorganization and the marked resistance which they display toward lack of food may be due to possession of both a mechanism for reserving the food stored in the tissues and to a power of rejuvenation which asserts itself when the process of starvation has, so to speak, "cleared the decks for action." At the present time, however, it is impossible to say definitely, whether or not the increase in metabolism which appears after 8 weeks' starvation, is a further insurance toward longevity or on the other hand, is a forerunner of death, being a result of the breaking down of the mechanism which has been depressing the rate of use of stored food.

That different species of fishes differ in their metabolic reaction to starvation is indicated by the results of a few experiments performed with starving bullheads (*Ameiurus melas* Raf.). With this species no stage was found where the starving individuals showed an increased resistance to low oxygen. Other experiments now under way may prove that the depression in metabolism in this species merely lasts for a shorter time than it does in the case of the more highly organized rock bass.

One further point should be considered in this discussion. It will be remembered (Fig. 1) that the normal resistance of the rock bass rises rapidly during the fall and winter months. We are at the same time accustomed to thinking of the breeding season in most animals as being a period of high metabolic activity and there is much evidence for this belief. It is, however, at the beginning of the breeding period that we find the fishes in question showing the greatest resistance to lack of oxygen. We have then a fact that tends to contradict what has gone before, for we have been proceeding upon the basis that animals with a low rate of metabolism are more resistant to lack of oxygen than are those with a higher rate. The explanation of this phenomenon is not at present clear but it may be that the contradiction is more apparent than real. The explanation of how a fish with a high rate of metabolism can be more resistant to lack of oxygen than one with a lower rate may be found perhaps, in a qualitative rather than a quantitative investigation of metabolism. Theories of anaërobic respiration suggest that there may be present in the fish, previous to the breeding season, large amounts of certain tissues that enter readily into the securing of an oxygen supply from some source other than the free oxygen. It is hoped that something may be done toward the solution of this question in the near future.

VI. Summary.

1. It has been shown that starvation in the rock bass (*Amblo-plites rupestris* Raf.) produces first a rapid and marked increase in the resistance of the starving fishes to lack of oxygen and N/25,000 KCN. Later this increase disappears and after 53 days of starvation the fishes that have been without food show a considerably lower resistance to lack of oxygen and to KCN solutions than do the controls. Furthermore, the starving fishes are now less resistant than are fishes that have gone without food for a shorter period.

2. The experiments with both lack of oxygen and with KCN give results that place the fishes midway between the mammals and the flat worms so far as the effects of starvation upon rate of metabolism are concerned. In flat worms starvation initiates and maintains an increased rate of metabolism up to death;

in the fishes starvation first initiates an increased rate of metabolism which later gives way to a decrease; in mammals starvation results in a depression of metabolism which depression continues up to death.

3. The experiments recorded here tend to show that there is a fundamental similarity in the physiological disorganization caused by lack of oxygen and KCN treatments. The meaning of this similarity was not determined.

4. There is an apparent contradiction in the results in that, just previous to the breeding season, when fishes in general, possess a high rate of metabolism, the seasonal resistance curve shows a much greater resistance to lack of oxygen and to KCN than at other seasons of the year. This contradiction is yet to be explained.

VII. Acknowledgments and Bibliography.

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